

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b>  <b>C07K 5/04, 5/06, A61K 37/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 93/09135</b>  <b>(43) International Publication Date:</b> 13 May 1993 (13.05.93)
<b>(21) International Application Number:</b> PCT/EP92/02472 <b>(22) International Filing Date:</b> 29 October 1992 (29.10.92)  <b>(30) Priority data:</b> 9123326.2                      4 November 1991 (04.11.91)    GB  <b>(71) Applicant (for AT only):</b> SANDOZ-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H. [AT/AT]; Brunner Strasse 59, A-1230 Vienna (AT).  <b>(71) Applicant (for DE only):</b> SANDOZ-PATENT-GMBH [DE/ DE]; Humboldtstrasse 3, D-7850 Lörrach (DE).  <b>(71) Applicant (for all designated States except AT DE US):</b> SAN- DOZ LTD. [CH/CH]; Lichtstrasse 35, CH-4002 Basle (CH).		<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> HENG, Richard [FR/ CH]; Landoltstrasse 63, CH-3006 Berne (CH). PAYNE, Trevor, Glyn [AU/CH]; Dalmazirain 26, CH-3005 Berne (CH). REVESZ, Laszlo [CH/CH]; ob dem Ficht- enrain 7, CH-4106 Therwil (CH). WEIDMANN, Beat [CH/CH]; Ulmenstrasse 12, CH-4123 Allschwil (CH).  <b>(74) Common Representative:</b> SANDOZ LTD.; Patents and Trademarks Div., Lichtstrasse 35, CH-4002 Basle (CH).  <b>(81) Designated States:</b> AU, CA, CS, FI, HU, JP, KR, NO, PL, RO, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PEPTIDES INHIBITING IL-1 BETA RELEASE  <b>(57) Abstract</b>  Di-, tri- and tetrapeptides in which the last $\alpha$ -amino acid is based on aspartic acid and attached to a residue $A_5$ which is H; CF <sub>3</sub> ; -Z <sub>1</sub> -Z <sub>2</sub> -Y <sub>2</sub> wherein each of Z <sub>1</sub> and Z <sub>2</sub> independently is a direct bond or an $\alpha$ -amino acid residue and Y <sub>2</sub> is NH <sub>2</sub> , C <sub>1-4</sub> alkylamino, di-(C <sub>1-4</sub> alkyl)amino or a heterocyclic radical attached by a nitrogen to Z <sub>2</sub> ; -CH <sub>2</sub> -X <sub>1</sub> -Y <sub>3</sub> wherein X <sub>1</sub> is O or S and Y <sub>3</sub> is heteroaryl; -CH <sub>2</sub> -Y <sub>3</sub> ; substituted phenyl; ring substituted phenoxymethylene or phenylthiomethylene; ring substituted pyridyloxymethylene; or a radical -CH <sub>2</sub> -X <sub>1</sub> -CO-Y <sub>4</sub> wherein X <sub>1</sub> is O or S and Y <sub>4</sub> is trialkylmethyl or substituted phenyl or pyridyl, in free form or in salt form, have pharmacological activity, e.g. IL-1 $\beta$ release inhibiting properties.		

**FOR THE PURPOSES OF INFORMATION ONLY**

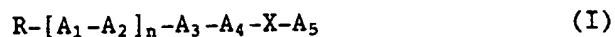
Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LJ	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

## PEPTIDES INHIBITING IL-1 BETA RELEASE

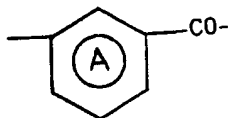
The present invention relates to peptides having pharmaceutical utility, processes for their production, pharmaceutical compositions comprising them and their use as pharmaceuticals.

More particularly the present invention provides a compound of formula I



wherein

- R is hydrogen, an amino protecting group or optionally ring substituted benzyloxy
- A<sub>1</sub> is Val, Leu, Ala, Ile or trimethylsilyl-Ala
- A<sub>2</sub> is Phe or Tyr,
- n is 0 or 1,
- A<sub>3</sub> is a direct bond, Val, Leu, Ala, Ile, trimethylsilyl-Ala or a divalent radical of formula (a)



(a)

wherein ring A is optionally substituted by hydroxy or C<sub>1-4</sub>alkoxy,

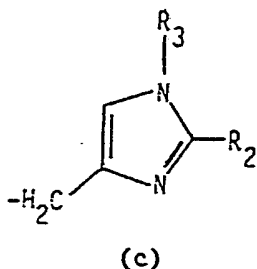
- 2 -

A<sub>4</sub> is a direct bond or a divalent radical of formula (b)

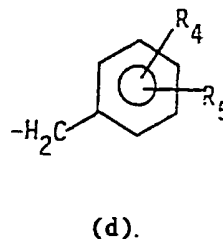


wherein R<sub>1</sub> is hydrogen or C<sub>1-4</sub>alkyl, and

Y<sub>1</sub> is the residue attaching to the α-carbon atom of an α-amino acid and optionally protected, -CH<sub>2</sub>-CH<sub>2</sub>-N(C<sub>1-4</sub>alkyl)<sub>2</sub>, imidazol-2-yl-methyl, benzimidazol-2-yl-methyl, 1H-1,2,4-triazol-3-yl-methyl, pyrazol-3-yl-methyl, indazol-3-yl-methyl or a radical of formula (c) or (d)



or

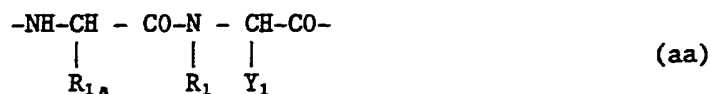


wherein

each of R<sub>2</sub> and R<sub>3</sub>, independently, is hydrogen, halogen, C<sub>1-4</sub>alkyl, CF<sub>3</sub> or trityl, at most one of R<sub>2</sub> and R<sub>3</sub> being H, and

each of R<sub>4</sub> and R<sub>5</sub> independently is hydrogen, C<sub>1-4</sub>alkyl, hydroxy, C<sub>1-4</sub>alkoxy, CF<sub>3</sub>, phenyl or halogen, at most one of R<sub>4</sub> and R<sub>5</sub> being H,

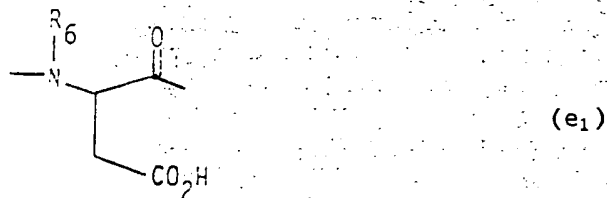
or A<sub>3</sub> and A<sub>4</sub> form together a radical of formula (aa)



wherein Y<sub>1</sub> is as defined above and R<sub>1</sub> and R<sub>1a</sub> form together -(CH<sub>2</sub>)<sub>m</sub>- wherein m is 2, 3, 4 or 5, and

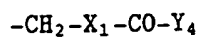
- 3 -

1) X is a divalent radical of formula (e<sub>1</sub>)

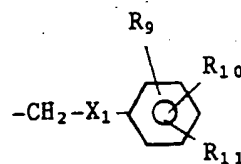


wherein R<sub>6</sub> is H or C<sub>1-4</sub>alkyl,

and A<sub>5</sub> is hydrogen; CF<sub>3</sub>; a radical -Z<sub>1</sub>-Z<sub>2</sub>-Y<sub>2</sub> wherein each of Z<sub>1</sub> and Z<sub>2</sub> independently is a direct bond or an α-amino acid residue and Y<sub>2</sub> is NH<sub>2</sub>, C<sub>1-4</sub>alkylamino, di-(C<sub>1-4</sub>alkyl)amino or a heterocyclic radical attached by a nitrogen to Z<sub>2</sub>; a radical -CH<sub>2</sub>-X<sub>1</sub>-Y<sub>3</sub> wherein X<sub>1</sub> is O or S and Y<sub>3</sub> is heteroaryl; a radical -CH<sub>2</sub>-Y<sub>3</sub>; or a radical of formulae (k) to (m)

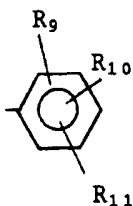


(k)

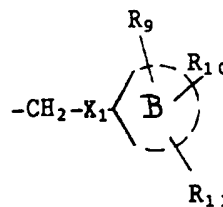


(l)

or



(m)

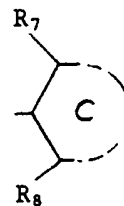


(o)

wherein

Y<sub>4</sub> is tri-(C<sub>1-4</sub>alkyl)methyl or a residue

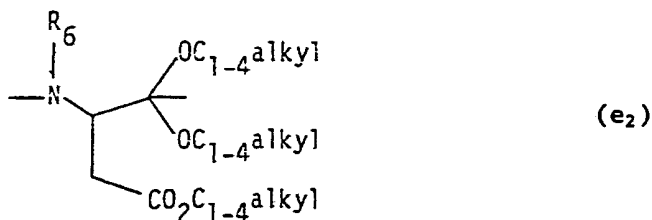
ring B is pyridyl,



- 4 -

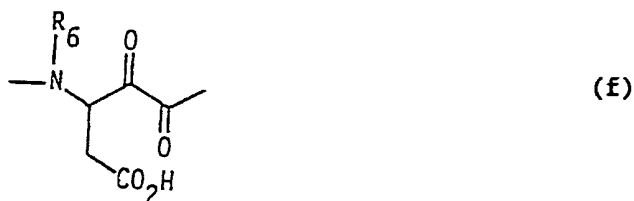
ring C is phenyl or pyridyl,  
 each of  $R_7$  and  $R_8$  independently is  $C_{1-4}$ alkyl,  $C_{1-4}$ alkoxy,  
 $CF_3$ , halogen, nitro or cyano, and  
 each of  $R_9$ ,  $R_{10}$  and  $R_{11}$  independently is nitro, cyano,  
 $CF_3$ , carbamoyl,  $CO_2R_{12}$ ,  $-CH=CH-CN$  or  $-CH=CHCO_2R_{12}$  wherein  
 $R_{12}$  is  $C_{1-6}$ alkyl,

X being also a divalent radical of formula ( $e_2$ )



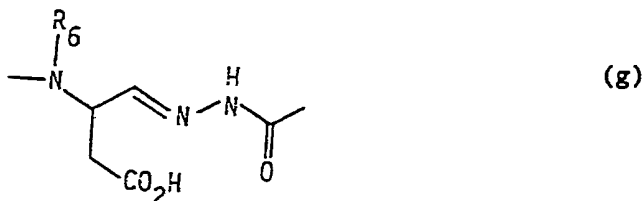
when  $A_5$  is H, or

2) X is a divalent radical of formula (f),



and  $A_5$  is  $-Z_1-Z_2-Y_2$  or a radical of formulae (k) to (o) as defined above, or  $OR_{13}$  or  $NR_{14}R_{15}$  wherein  $R_{13}$  is  $C_{1-12}$ alkyl optionally substituted by OH or interrupted by O and each of  $R_{14}$  and  $R_{15}$  is independently hydrogen,  $C_{1-12}$ alkyl,  $C_5-7$ cycloalkyl or benzyl, or

3) X is a divalent radical of formula (g)

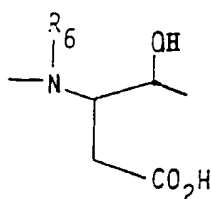




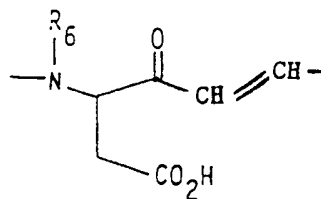
- 5 -

and  $A_5$  is  $-Z_1-Z_2-Y_2$  as defined above, or

4) X is a divalent radical of formula (h) or (j)



(h)



(j)

and  $A_5$  is a radical of formulae (k) to (o),  $-\text{CH}_2-\text{Y}_3$  or  $-\text{CH}_2-\text{X}_1-\text{Y}_3$  as defined above,

with the provisos that

only one of  $A_3$  and  $A_4$  can be a direct bond when  $n$  is 0, and each of  $A_3$  and  $A_4$  is other than a direct bond when  $n$  is 1.

and the physiologically-hydrolysable and -acceptable esters or amides thereof,

in free form, in salt form or in the form of complexes.

Examples of protecting groups as R are e.g. disclosed in "Protective Groups in Organic Synthesis", T. W. Greene, J. Wiley & Sons NY (1981), 219-287, for example acyl such as acetyl, methoxy-succinyl, hydroxysuccinyl or benzoyl optionally substituted on the phenyl ring with e.g. p-methoxycarbonyl, p-methoxy or p-nitro; alkoxycarbonyl such as t-butyloxycarbonyl; arylmethoxycarbonyl such as 9-fluorenylmethoxycarbonyl or benzyloxy carbonyl optionally substituted on the phenyl ring with p-methoxy, p-nitro, p-chloro or m-phenyl; arylmethyl such as benzyl optionally ring substituted with p-methoxy, p-nitro or p-chloro; or arylsulfonyl such as phenylsulfonyl optionally ring substituted

- 6 -

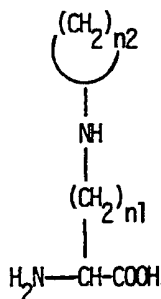
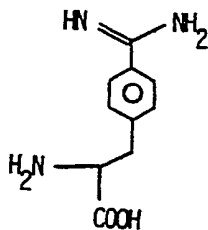
with p-methyl or p-methoxy, or naphthylsulfonyl optionally ring substituted with e.g. amino or di(C<sub>1-4</sub>alkyl)amino.

When R is ring substituted benzyloxy, it is preferably benzyloxy substituted with hydroxy or C<sub>1-4</sub>alkoxy. Preferably R is unsubstituted benzyloxy.

Halogen is preferably fluorine or chlorine.

When A<sub>3</sub> is a substituted radical of formula (a) it is preferably substituted by C<sub>1-4</sub>alkoxy, preferably in para to -C=O.

By  $\alpha$ -amino acid is meant a naturally occurring or commercially available or non natural  $\alpha$ -amino acid or an optical isomer thereof. A non natural  $\alpha$ -amino acid is an  $\alpha$ -amino acid which is not incorporated into a protein under mRNA direction, e.g.  $\beta$ -Nal, a fluoro- $\alpha$ -amino acid such as fluoroalanine, trimethylsilyl-Ala or an  $\alpha$ -amino acid such as



wherein  $n_1$  is an integer from 1 to 6 and  $n_2$  is an integer from 1 to 12.

Protecting groups which may be present in Y<sub>1</sub> are groups which protect the O, S or N functionality in the side chain amino groups of an  $\alpha$ -amino acid. N-protecting groups are e.g. as disclosed above for R, or C<sub>3-5</sub>alkyl such as isopropyl, formyl, a sugar residue such as 1-deoxy-fructosyl or  $\alpha$ -glucosyl(1-4)-

deoxyfructosyl, dihydroxy- $C_{3-6}$ alkyl such as dihydroxypropyl,  $C_{5-7}$ cycloalkyl such as cyclohexyl or tropinyl. O- and S-protecting groups for hydroxy and thiol functionalities are known and may be e.g. methyl, t.-butyl or benzyl.

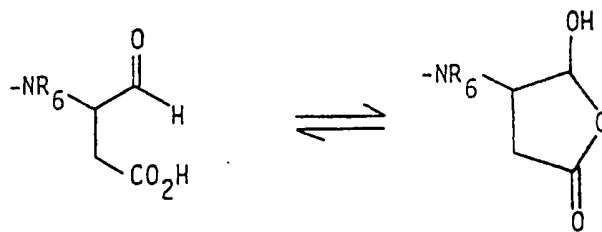
When  $Y_2$  is a heterocyclic radical, it may be e.g. a 5 or 6 membered ring, e.g. piperidino or pyrrolidinyl.

Examples of heteroaryl as  $Y_3$  include e.g. 5-, 6- or 7-membered unsaturated heterocyclic radicals, comprising at least one nitrogen and optionally further heteroatoms such as N, O or S. Preferably  $Y_3$  is heteroaryl comprising from 1 to 4 nitrogen atoms, e.g. pyridyl, triazolyl, tetrazolyl, triazin-dionyl.

In ring B of radical (o), the nitrogen atom may be in o-, m- or para. When ring C in radical (k) is pyridyl, it may be 3-, 4- or 5-pyridyl.

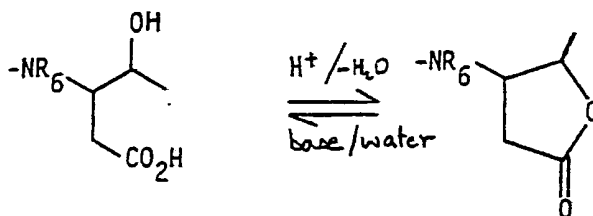
Radicals ( $e_1$ ), ( $e_2$ ), (f), (g) and (j) are derived from Asp and comprise one asymmetric carbon atom and radical (h) comprises two asymmetric carbon atoms and accordingly they lead to optical isomerism. It will be understood that the present invention includes all individual isomeric forms and diastereoisomers as well as mixtures, e.g. racemates, unless otherwise stated.

Radical of formula ( $e_1$ ) attached to  $A_5$  which is hydrogen may exist in both cyclic as well as in non-cyclic form e.g. as follows:



It is to be understood that where tautomeric forms occur, the present invention embraces both lactol and oxo-carboxylic acid forms, i.e. although compounds of formula I wherein X is a radical of formula (e<sub>1</sub>) are defined for convenience by reference to the oxo-carboxylic acid form only, the invention is not to be understood as being in any way limited by the particular nomenclature or graphic representation employed. Similar considerations apply in relation to starting materials exhibiting lactol/oxo-carboxylic acid tautomerism as hereinafter described.

The same considerations also apply to radical of formula (h) which may exist in both linear and cyclic form as follows:



and to compounds of formula I comprising a radical of formula (h) and to the corresponding starting materials.

By the term "physiologically-hydrolysable and -acceptable esters or amides" are meant esters and amides which are hydrolysable under physiological conditions to yield alcohols or amines which are themselves physiologically acceptable, i.e. which are non-toxic at the desired dosage levels.

Such esters or amides are obtained by esterification or amidation, respectively, of a compound of formula I wherein X is a radical bearing a carboxy group. Such esters include esters with an aliphatic or alicyclic alcohol or polyol having 1 to 12 carbon atoms. Such amides include amides with aliphatic amines, e.g. C<sub>1-4</sub>alkyl amine, C<sub>1-4</sub>alkoxy-C<sub>1-4</sub>alkyl amine such as

$\beta$ -methoxy-ethyl amine, or aniline.

The compounds of formula I may exist e.g. in free form, acid addition salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Salt forms may also include those obtainable with the carboxylic group present in compounds of formula I, e.g. alkali metal salts such as sodium or potassium, or substituted or unsubstituted ammonium salts. Complexes are e.g. formed from compounds of formula I on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or an addition of polymeric organic substances.

In the compounds of formula I, the following significances are preferred either individually or in any combination or sub-combination:

1. R is an amino protecting group or benzyloxy, preferably an amino protecting group. R is preferably benzyloxy when n is 0 and A<sub>3</sub> is a radical of formula (a).
2. Each of A<sub>3</sub> and A<sub>4</sub> are other than a direct bond.
3. n is 0.
4. A<sub>3</sub> is a radical of formula (a) when n is 0.
5. A<sub>3</sub> is a direct bond, Val, Leu, Ala, Ile or trimethylsilyl-Ala.
6. R<sub>1</sub> is hydrogen or methyl, preferably hydrogen.
7. Y<sub>1</sub> is the residue attaching to the  $\alpha$ -carbon atom of an  $\alpha$ -amino acid selected from Ala, Leu, His, Phe, Met, Trp, trimethylsilyl-Ala and optionally side chain protected Arg, Orn and Lys, or Y<sub>1</sub> is a radical of formula (c).

- 10 -

8.  $A_3$  and  $A_4$  may also form together a radical of formula (aa) when  $n$  is 0.
9.  $m$  is 2 or 3.
10.  $X$  is a radical of formula ( $e_1$ ) and  $A_5$  is other than H.
11.  $X$  is a radical of formula ( $g$ ).
12.  $X$  is a radical of formula ( $h$ ) or ( $j$ ).
13.  $R_6$  is hydrogen or methyl, preferably hydrogen.
14.  $Z_1$  in  $A_5$  is the residue of a natural  $\alpha$ -amino acid, preferably of an aromatic/heterocyclic  $\alpha$ -amino acid, particularly Pro.
15.  $Z_2$  in  $A_5$  is the residue of a natural  $\alpha$ -amino acid, preferably an aliphatic  $\alpha$ -amino acid, particularly an aliphatic  $\alpha$ -amino acid without further functional group, most preferably Val.
16.  $X$  is a radical of formula ( $e_1$ ) optionally esterified or amidated and  $A_5$  is a radical of formula ( $k$ ), ( $l$ ) or ( $o$ ).
17.  $X$  is a radical of formula ( $e_1$ ), ( $h$ ) or ( $j$ ) optionally esterified or amidated and  $A_5$  is  $-\text{CH}_2-\text{X}_1-\text{Y}_3$ , preferably  $-\text{CH}_2-\text{S}-\text{Y}_3$ , or  $-\text{CH}_2-\text{Y}_3$ .
18.  $X$  is a radical of formula ( $h$ ) or ( $j$ ) optionally esterified or amidated and  $A_5$  is ( $k$ ).
19.  $X$  is a radical of formula ( $j$ ) optionally esterified or amidated and  $A_5$  is a radical of formula ( $m$ ).
20. Radical of formula ( $m$ ) is monosubstituted, preferably in

para, more preferably it is 4-nitrophenyl.

21. All  $\alpha$ -amino acid residues present except X have the L configuration. X has the D or L configuration.
22. The asymmetric carbon in (h) bearing the OH group has the configuration S. Preferably the asymmetric carbon in (j) bearing  $R_6$  has the configuration R.

In a series of specific embodiments, the present invention also provides a compound of formula I wherein n is 0,  $A_3$  is a direct bond, Val, Leu, Ala, Ile or trimethylsilyl-Ala,  $A_4$  is as defined above, or  $A_3$  and  $A_4$  form together a radical of formula (aa) as defined above, and

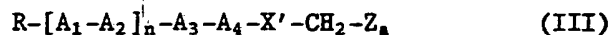
- i) X is a radical of formula ( $e_1$ ) or ( $e_2$ ) as defined above and  $A_5$  is H, or
- ii) X is a radical of formula ( $e_1$ ) or (f) and  $A_5$  is  $-Z_1-Z_2-Y_2$  as defined above or a radical of formula (k), (l) or (m) wherein  $X_1$  is O,
- iii) X is a radical of formula (g) and  $A_5$  is  $-Z_1-Z_2-Y_2$ , or
- iv) X is a radical of formula (j) and  $A_5$  is a radical of formula (k), (l) or (m) wherein  $X_1$  is O.

The present invention also provides a process for the production of a compound of formula I, which process comprises:

- a) removing at least one protecting group from a compound of formula I in protected form or adding a protecting group R at the N-terminal group of a compound of formula I; or
- b) converting one compound of formula I into another compound of formula I; or
- c) coupling together by an amide bond two peptide fragments,

each of which contains at least one amino acid in protected or unprotected form and one peptide fragment containing a radical of formula (e<sub>1</sub>) to (j) as defined above, the peptide fragments being such that a protected or unprotected peptide having the sequence according to formula I above is obtained and, if necessary, removing the protecting group or groups from a compound of formula I in protected form; or

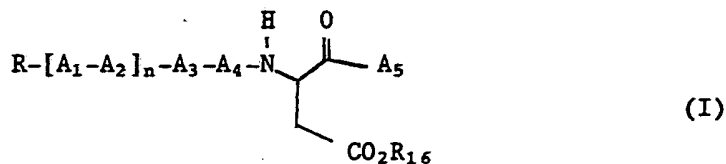
- d) for the production of a compound of formula I wherein X is a radical of formula (e<sub>1</sub>) or (h) and A<sub>5</sub> is a radical of formula (k), (l) or (o) or -CH<sub>2</sub>-X<sub>1</sub>-Y<sub>3</sub>, reacting a compound of formula III



wherein R, A<sub>1</sub> to A<sub>4</sub> and n are as defined above, X' is a radical of formula (e<sub>1</sub>) or (h), and Z<sub>a</sub> is a leaving group, e.g. halogen,

with a corresponding phenol, thiophenol or HX<sub>1</sub>-pyridine or an acid of formula HX<sub>1</sub>-CO-Y<sub>4</sub> or a functional derivative thereof or HX<sub>1</sub>-Y<sub>3</sub>; or

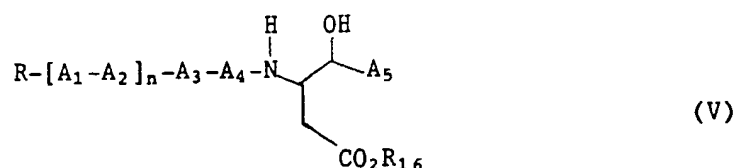
- e) for the production of a compound of formula I



wherein R, A<sub>1</sub> to A<sub>5</sub> and n are as defined above and R<sub>16</sub> is a C<sub>1-12</sub> aliphatic or alicyclic residue, oxidizing a compound of formula V



- 13 -



wherein R, A<sub>1</sub> to A<sub>5</sub>, n and R<sub>16</sub> are as defined above,

and recovering a compound of formula I thus obtained in free or salt form or in the form of a complex.

Processes (a) to (e) above may be carried out in accordance with standard techniques known in the art.

The removal of a protecting group in process step (a) may also include the removal of R on the N-terminal group of a compound of formula I. For example, when R is benzyloxy carbonyl, this group may be removed by hydrogenation in the presence of a catalyst, e.g. Pd.

In accordance with process step (b) for example, for the production of a compound of formula I wherein X comprises a carboxy group, a compound of formula I wherein X comprises an esterified or amidated carboxy group may be hydrolysed. Such hydrolysis may be effected by treatment with an appropriate alkali or by acid hydrolysis, for example in the presence of trifluoroacetic acid.

Furthermore, in accordance with process step (b), for the production of a compound of formula I wherein X comprises an esterified or amidated carboxy group, a compound of formula I wherein X comprises a carboxy group or an esterified carboxy group may be (trans) esterified or amidated. Such ester formation or amidation may be carried out using any of the techniques known in the art, for example converting the carboxy group in a functional

- 14 -

reactive group, e.g. a corresponding carbonyl halide or anhydride, or using a compound of formula I wherein X is a radical of formula (h) in the lactone form, and reacting such group with the selected alcohol or amine.

In accordance with a further embodiment of process step (b), for the production of a compound of formula I wherein X is a radical of formula (g), a compound of formula I wherein X is a radical of formula (e<sub>1</sub>) or (e<sub>2</sub>) and A<sub>5</sub> is H may be reacted with a compound of formula II



wherein A<sub>5</sub> is as defined above. This process may be carried out in analogy to the known techniques used for the preparation of semi carbazones.

A compound of formula I wherein X is a radical of formula (e<sub>1</sub>) may also be converted in accordance with known techniques into a compound of formula I wherein X is a radical of formula (e<sub>2</sub>) and vice versa.

Process step (c) may be carried out by the techniques known in the art of peptide chemistry. By peptide fragment comprising a radical of formula (e<sub>1</sub>) to (j) is also meant the radical itself bearing a protecting group on the -NR<sub>6</sub>- moiety and an appropriate ending, e.g. A<sub>5</sub> or CH<sub>2</sub>-Z<sub>a</sub>, on the other end.

Process step (d) may conveniently be effected using a Dess-Martin reagent, e.g. in the presence of a base or a halogen-precipitating silver salt, or according to the Swern oxidation procedures.

Where desired, in these reactions, protecting groups may be used for functional groups which do not participate in the reaction.

These may be e.g. amino protecting groups, carboxy protecting groups, acetal groups etc. When the desired reaction is complete, the protecting groups may then be removed.

Each of the above processes may be carried out using starting materials in the form of one or other of the individual optical isomers or in the form of mixtures [relating to the asymmetric carbons present in radicals of formulae (e<sub>1</sub>) to (j) as X or in such a radical precursor]. Conveniently the starting materials are used as S- or R-enantiomers to produce a compound of formula I wherein the asymmetric carbon in radicals of formulae (e<sub>1</sub>) to (j) has the S or R configuration, respectively.

The starting materials used in process steps (d) or (e) may be prepared in analogy with process step (c).

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known and practiced in the art.

The following examples are illustrative of the invention. All temperatures are in °C.

The following abbreviations are used:

THF = tetrahydrofuran  
TFA = trifluoroacetic acid  
MeOH = methanol  
EtOAc = ethyl acetate  
DCC = dicyclohexylcarbodiimide  
HOBT = hydroxybenzotriazole  
Z = benzyloxycarbonyl  
r.t. = room temperature  
Fmoc = 9-fluorenylmethoxycarbonyl  
a = amorph

EXAMPLE 1: Z-Val-Met-Asp(OH)-H

Z-Val-Met-Aspartic aldehyde dimethyl acetal- $\beta$ -tert.butyl ester (1.17 g) is taken up in  $\text{CH}_2\text{Cl}_2$  (150 ml), TFA (15 ml) and water (1 ml) added and stirred for 2,5 hrs at r.t. The solvent is evaporated, toluene added twice and evaporated again. The crystalline residue is dissolved in water (60 ml) upon heating, decanted from some undissolved material and crystallized at 5 °, affording the first crop of the product: m.p. 117 °. All mother liquors are collected and chromatographed ( $\text{SiO}_2$ , acetone/hexane/-EtOAc 60/40/0.5) to give a second crop of product, crystallizing upon trituration with water.

EXAMPLE 2: Z-Val-Met-Asp(OH)-H Semicarbazone

Compound of Example 1 (0.27 g, 0.55 mmol) in MeOH (2 ml) is combined with semicarbazide, HCl (0.6 ml of 1 molar solution), then 5 drops of pyridine are added. The product crystallizes after 10 min. at r.t.: m.p. 233 - 235 °.

EXAMPLE 3: Z-Val-Met-Asp(OH)-H semicarbazonyl-Pro-Val-N(CH<sub>3</sub>)<sub>2</sub>

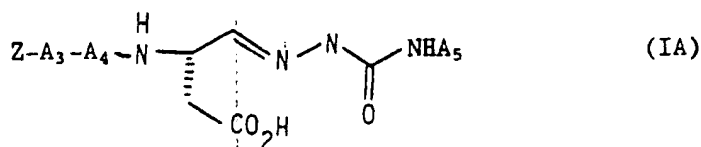
Compound of Example 1 (0.48 g, 1 mmol) is dissolved in a mixture of MeOH (2 ml) water (0.5 ml) and 3 drops of pyridine. N-(hydrazinocarbonyl)-Pro-Val-N(CH<sub>3</sub>)<sub>2</sub> (0.3 g, 1 mmol) is dissolved in MeOH (1 ml), water (2 ml) and 3 drops of 2N HCl. Both solutions are combined and warmed for 2 min. at 40 - 50 °. The product crystallizes upon cooling: m.p. 118 - 120 °.

By repeating the procedure of Example 1, using the corresponding starting materials, following compounds may be prepared:

Example 4                      Z-Val-Phe-Asp(OH)-H

**Example 5**                      Z-Val-His-Asp(OH)-H

By repeating the procedure of Example 2 or 3 respectively, using the corresponding starting materials, following compounds of formula IA



wherein A<sub>3</sub>, A<sub>4</sub> and A<sub>5</sub> are as defined below, may be prepared.

Example	A <sub>3</sub>	A <sub>4</sub>	A <sub>5</sub>	M.P. °C
6	Val	Phe	H	a
7	Val	His	H	150
8	Val	Phe	Pro-Val-N(CH <sub>3</sub> ) <sub>2</sub>	126-129
9	Val	His	Pro-Val-N(CH <sub>3</sub> ) <sub>2</sub>	
10	Ala-Tyr-Val	Phe	H	205

**EXAMPLE 11: (3S)-3-(Z-valyl-phenylalanyl)-amino-5-(2,6-dimethylbenzoyloxy)-4-oxo pentanoic acid**

(3S,4RS)-3-(Z-Val-His)amino-4-hydroxy-5-(2,6-dimethylbenzoyloxy) pentanoic acid tert.-butyl ester (0.6 mmol) is treated with Dess-Martin reagent (0.77 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 ml) for 45 min. and filtered. Aceton and 0.5 N NaOH are then added and aceton is evaporated. The residual crystalline material is washed with H<sub>2</sub>O, acidified with 10 % tartaric acid and extracted with EtOAc. The combined EtOAc extracts affords after evaporation the tert. butyl ester of the product as yellow crystals. These are dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 ml), TFA (4 ml) is added and the mixture is stirred at r.t. for 15 min. The mixture is evaporated to dryness and chromatographed (SiO<sub>2</sub>, aceton/hexane/EtOAc 60/40/1) affording the product as slightly colored crystals.

EXAMPLE 12: (Z-valyl-alanyl)-(3R,4S)-3-amino-4-hydroxy-5-(2,6-dichlorobenzoyloxy)

(Z-valyl-alanyl)-3R,4S)-3-amino-4,5-dihydroxy pentanoic acid ethyl ester and 4-dimethylaminopyridine are dissolved in pyridine and 2,6-dichlorobenzoyl chloride is added dropwise. The reaction mixture is stirred overnight at room temperature, then ice and water are added and the mixture is extracted with AcOEt. The organic layer is washed with water, then with NaCl solution, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, evaporated, and the crude product is chromatographed to give the title compound.

The (3R,4RS) derivative of the title compound may be prepared as follows:

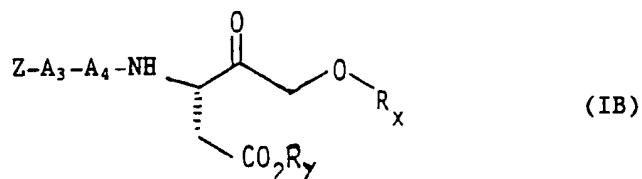
Z-Val-Ala-OH (0.26 mmol), DCC (53 mg, 0.26 mmol) and HOBT.H<sub>2</sub>O (39 mg, 0.26 mmol) are dissolved in THF/DMF (2 ml/2 ml) and stirred for 5 min., before (3S,4RS)-3-amino-4-hydroxy-5-(2,6-dimethylbenzoyloxy)-pentanoic acid tert.butyl ester (87 mg, 0.26 mmol) in THF (2 ml) is added. The reaction mixture is stirred overnight at r.t., evaporated and chromatographed (SiO<sub>2</sub>, EtOAc/MeOH/NH<sub>3</sub> 95/5/0.5) to afford the (3R,4RS) title compound.

EXAMPLE 13: (Z-valyl-alanyl)-(3R)-3-amino-4-oxo-5-(2,6-dichlorobenzoyloxy) pentanoic acid ethyl ester

Dimethylsulfoxide in CH<sub>2</sub>Cl<sub>2</sub> is added dropwise to a solution of oxalyl chloride in CH<sub>2</sub>Cl<sub>2</sub> at -50°C. After 15 min., a solution of compound of Example 12 in CH<sub>2</sub>Cl<sub>2</sub> is added dropwise and the reaction mixture stirred for 1 hour at -40°C. Triethylamine is added and stirring is continued for 3 hours at room temperature. Water is then added and the reaction mixture extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer is washed with NaHCO<sub>3</sub> solution and

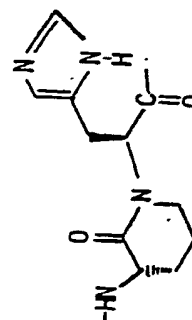
NaCl solution, dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated. The residue is chromatographed yielding the title compound.

By repeating the procedure of Examples 1-3 and 11 to 13, using the corresponding starting materials, following compounds of formula IB

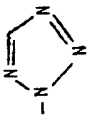
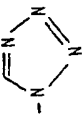
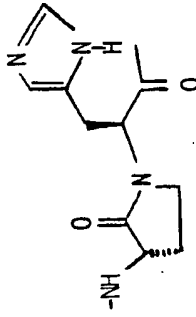


wherein  $\text{A}_3$ ,  $\text{A}_4$ ,  $\text{R}_x$  and  $\text{R}_y$  are as defined below, may be prepared.

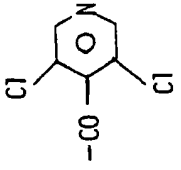
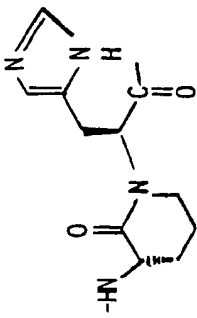
EXAMPLE	A <sub>3</sub>	A <sub>4</sub>	R <sub>y</sub>	R <sub>x</sub>	[α] <sub>D</sub> <sup>20</sup> or M.P.	c (MeOH) °C (2)
14	Val	Phe	H	2,6-diMe-benzoyl		
15	Val	His	H	2,6-diMe-benzoyl		
16	Val	His	H	p-NO <sub>2</sub> -phenyl		
17	Val	His	H	H	a (2)	
		(1-trityl)				
18	Val	His	H	2,6-diCl-benzoyl		
19	Val	His	H	2,6-diCF <sub>3</sub> -benzoyl		
20	Val	His	H	2,6-diNO <sub>2</sub> -benzoyl		
21	Val	His	H	2,6-diCF <sub>3</sub> -benzoyl		
		(2-CF <sub>3</sub> )				
22	Val	Trp	H	2,6-diCF <sub>3</sub> -benzoyl		
23	TriMe-silyl- Ala	His	H	2,6-diCl-benzoyl		
24			H	2,6-diCl-benzoyl		





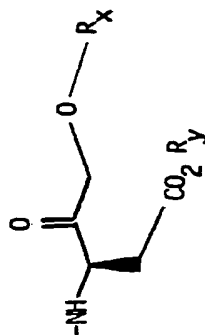
EXAMPLE	A <sub>3</sub>	A <sub>4</sub>	R <sub>y</sub>	R <sub>x</sub>	[α] <sub>D</sub> <sup>20</sup> or M.P.	c(MeOH) °C (2)
25	Ala-Tyr-Val	Phe	H	H	a (2)	
26	D-Val	His	H	2,6-diCl-benzoyl	-39.2°	1.21
27	Val	Arg	H	2,6-diCl-benzoyl		
28	Val	His	C <sub>2</sub> H <sub>5</sub>	2,6-diCl-benzoyl	-26.7°	1.02
29	Val	His	H		-18.0°	0.97
30	Val	His	H		-19.0°	0.96
31			H	2,6-diCl-benzoyl		

EXAMPLE	A <sub>3</sub>	A <sub>4</sub>	R <sub>y</sub>	R <sub>x</sub>	[α] <sub>D</sub> <sup>20</sup> or M.P.	c(MeOH) °C (2)
32	Val	Orn	H	2,6-diCl-benzoyl		
33	Val	Lys	H	2,6-diCl-benzoyl		
34	Val	Ala	H	2,6-diCl-benzoyl		
35	Val	His	H	Pivaloyl		
36	Val	His	i.C <sub>4</sub> H <sub>9</sub>	2,6-diCl-benzoyl	**	
37	Val	His	n.C <sub>10</sub> H <sub>21</sub>	2,6-diCl-benzoyl	**	
38	Val	His	benzyl	2,6-diCl-benzoyl	**	
39	Val	His	CH <sub>2</sub> CH <sub>2</sub> OH	2,6-diCl-benzoyl	**	
40	Val	Ala	C <sub>2</sub> H <sub>5</sub>	2,6-diCl-benzoyl	**	
41	Val	Leu	H	2,6-diCl-benzoyl		
42	Val	His	H	2,6-diF-benzoyl		
43	Val	His	H	2,6-diCl-benzoyl		
		(2-CF <sub>3</sub> )				
44	Val	His	C <sub>2</sub> H <sub>5</sub>	Pivaloyl	**	
45	Val	His	H	2,6-diCl-benzoyl	*	
46	Val	Lys	H	2,6-diCl-benzoyl		
		(N <sup>e</sup> -iC <sub>3</sub> H <sub>7</sub> )				
47	Val	Lys	C <sub>2</sub> H <sub>5</sub>	2,6-diCl-benzoyl	**	
		(N <sup>e</sup> -iC <sub>3</sub> H <sub>7</sub> )				
48	Val	Ala	H	2,6-diCl-benzoyl	**	
49	Val	Ala	t.C <sub>4</sub> H <sub>9</sub>	2,6-diCl-benzoyl	**	

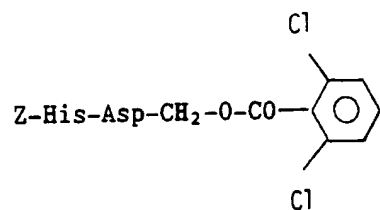
EXAMPLE	A <sub>3</sub>	A <sub>4</sub>	R <sub>Y</sub>	R <sub>X</sub>	[α] <sub>D</sub> <sup>20</sup> or M.P.	c(MeOH) °C (2)
50	Val	Ala	C <sub>2</sub> H <sub>5</sub>	2,6-diCl-benzoyl	*	*
51	Val	Ala	C <sub>2</sub> H <sub>5</sub>		*	*
52	TriMe-silyl- Ala	His	C <sub>2</sub> H <sub>5</sub>	2,6-diCl-benzoyl		
53			C <sub>2</sub> H <sub>5</sub>	2,6-diCl-benzoyl	**	**
54	Val	Ala	i.C <sub>3</sub> H <sub>7</sub>			**
55	TriMe-silyl- Ala	Ala	t.C <sub>4</sub> H <sub>9</sub>	2,6-diCl-benzoyl		
56	TriMe-silyl- Ala	Ala	H	2,6-diCl-benzoyl		

EXAMPLE	A <sub>3</sub>	A <sub>4</sub>	R <sub>y</sub>	R <sub>x</sub>	$[\alpha]_D^{20}$ or M.P.	c(MeOH) °C (2)
57	TriMe-silyl Ala	Ala	C <sub>2</sub> H <sub>5</sub>	2,6-diCl-benzoyl		

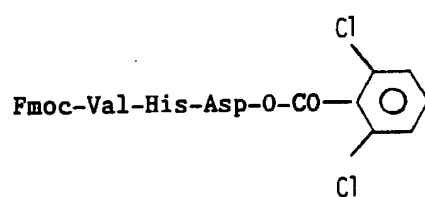
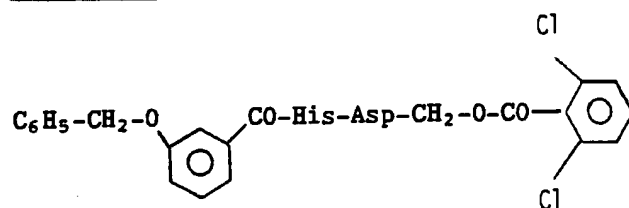
\* the compounds have the following configuration in the aspartyl moiety:



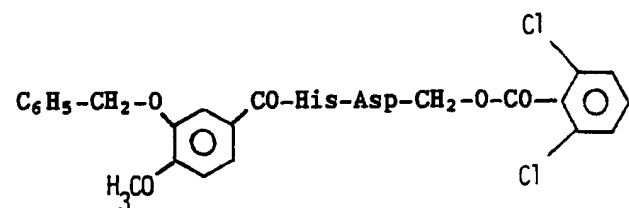
\*\* the compounds have the R,S configuration in the aspartyl moiety.

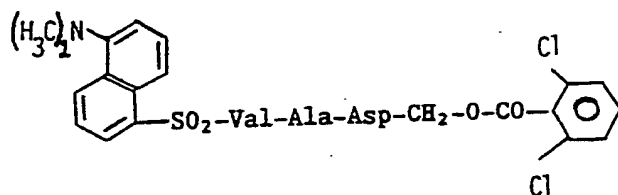
EXAMPLE 58:

$$[\alpha]_D^{20} = -20.3^\circ \quad c = 1 \text{ in MeOH}$$

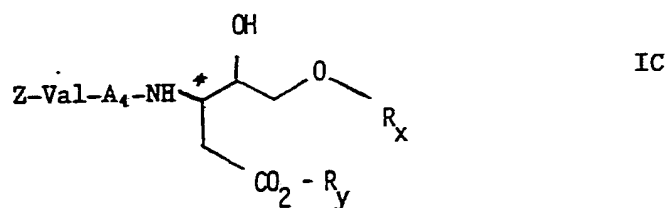
EXAMPLE 59:EXAMPLE 60:

$$[\alpha]_D^{20} = -21.5^\circ \quad c = 1.07 \text{ MeOH}$$

EXAMPLE 61:

EXAMPLE 62:

By repeating the procedure of Examples 1-3 and 12-13 and using the corresponding starting materials, following compounds of formula IC



wherein  $A_3$ ,  $R_x$  and  $R_y$  are as defined below, may be prepared

EXAMPLE	$A_4$	$R_y$	$R_x$	*
63	Ala	H	2,6-diCl-benzoyl	$R, S$
64	Ala	$C_2H_5$		$R$
65	Ala	$t.C_4H_9$	2,6-diCl-benzoyl	$R, S$
66	Ala	$-C_2H_4-OCH_3$	2,6-diCl-benzoyl	$R, S$

**EXAMPLE 67:** (3RS)-3-(Z-valyl-phenylalanyl)amino-4-oxo-6-(p-nitrophenyl)-transhex-5-enoic acid tert.butyl

(3RS)-3-(Z-Valyl-phenylalanyl)amino-4-oxo-pentanoic acid tert.butyl ester-5-diethylphosphonate (0.2 g, 0.29 mM) is dissolved in THF (15 ml) at 5°C. NaH (25 mg, 0.59 mM) is added and the mixture stirred for 10 min. p-Nitrobenzaldehyde (0.18 g, 1.1 mM) in THF (2 ml) is added at 0°C and the reaction mixture stirred for 45 min. at this temperature. The reaction mixture is poored on 5 % tartaric acid, extracted with ethyl acetate, the organic phases dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated and the product purified by chromatography yielding the title compound.

**EXAMPLE 68:** Z-Val-Phe-Aspartic aldehyde dimethyl acetal-β-methyl ester

Z-Val-Phe-Asp(OH)-H (0.8 g) is dissolved in MeOH (25 ml) containing 8 % HCl, left at r.t. over night, evaporated to dryness and the residual crystals washed with ether, providing the title compound as white crystals. M.p.: 168 - 171 °.

Starting materials may be prepared as follows:

**EXAMPLE 69:** (3S)-3-(Fluorenylmethoxycarbonyl)amino-5-iodo-4-oxobutanoic acid tert.butyl ester

Triethylamine (1.3 ml, 9.6 mmol), followed be ethylchloroformiate (0.92 ml, 9.6 mmol) are added to (3S)-3-(fluorenylmethoxycarbonyl)amino-3-carboxy-butanoic acid tert.butyl ester (3.5 g, 8.5 mmol) in THF (60 ml) at - 10 °. After 10 min., a solution of diazomethane in ether is added slowly, and the reaction mixture stirred for 45 min. at 0 - 5 °. HCl (2N) in ether is added at 5 - 10 ° until gas evolution has ceased. The reaction mixture is evaporated to dryness, taken up in acetone (50 ml) NaI (4 g) added

and stirred for 1 hr at r.t. Ether (150 ml) is added, the reaction mixture filtered and evaporated. The residue is chromatographed ( $\text{SiO}_2$ , EtOAc/hexane), yielding the title compound as slightly yellow crystals.

**EXAMPLE 70:** (3S)-3-(Fluorenylmethoxycarbonyl)amino-5(2,6-dimethylbenzoyloxy)-4-oxo pentanoic acid tert.butyl ester

3S-3-(Fluorenylmethoxycarbonyl)amino-5-iodo-4-oxo-butanoic acid tert.butyl ester (1 g, 1.8 mmol), 2,6-dimethylbenzoic acid (0.5 g, 3.3 mmol) and AgOAc (0.6 g, 3.6 mmol) are dissolved in acetone (25 ml) and refluxed for 1 hr. After filtration and evaporation the crude product is chromatographed ( $\text{SiO}_2$ , ether/hexane 3/7) yielding the title compound.

**EXAMPLE 71:** (3S)-3-Fluorenylmethoxycarbonyl)amino-5(4-nitrophenoxy)-4-oxo pentanoic acid tert.butyl ester

3S-3-(Fluorenylmethoxycarbonyl)amino-5-iodo-4-oxo-butanoic acid tert.butyl ester (1 g, 1.8 mmol), p-nitrophenol (0.5 g, 3.7 mmol) and  $\text{K}_2\text{CO}_3$  (0.38 g, 2.8 mmol) are refluxed in acetone (6 ml) for 30 min.,  $\text{CH}_2\text{Cl}_2$  added and the organic phase washed with 2N  $\text{NaHCO}_3$ . The combined organic phases are dried, evaporated and chromatographed ( $\text{SiO}_2$ , EtOAc/hexane 2/8), yielding the title compound as a yellow oil.

**EXAMPLE 72:** (Z-valyl-alanyl)-(3R,4S)-3-amino-4,5-dihydroxy pentanoic acid ethyl ester

a) Ethyl (3R,4S)-3-benzylamino-4,5-(isopropylidenedioxy) pentanoate (Y. Yamada, Tetrahedron Letters 1983, 24, 3009) and 10% Pd/C in ethanol are shaken at r.t. for 30 mn under  $\text{H}_2$ .



Filtration and evaporation of the reaction mixture give ethyl (3R,4S)-3-amino-4,5-(isopropylidenedioxy)pentanoate which is used without further purification.

- b) Z-Val-Ala-OH is dissolved in THF, HOBT·H<sub>2</sub>O and DCC are added at 5°C. After stirring for 20 min. at 5°C, diisopropylethylamine and ethyl (3R,4S)-3-amino-4,5-(isopropylidenedioxy)pentanoate in THF are added. Reaction mixture is stirred overnight at room temperature, filtered, evaporated and chromatographed to afford (Z-valyl-alanyl)-(3R,4S)-3-amino-4,5-(isopropylidenedioxy)pentanoic acid ethyl ester.
- c) Compound 74 b) is dissolved in AcOH/H<sub>2</sub>O (75/25) and stirred at 40°C for 4 hours. After evaporation, water is added and the mixture is extracted with AcOEt. The combined extracts are washed with water, NaHCO<sub>3</sub> solution, NaCl solution, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated, yielding the title compound.

The compounds of formula I their physiologically-hydrolysable and -acceptable esters and amides and their pharmaceutically acceptable salts (hereinafter referred to as compounds of the invention) exhibit pharmaceutical activity and are, therefore, useful as pharmaceuticals.

In particular, the compounds of the invention inhibit IL-1 $\beta$  secretion as indicated in the following in vitro test using THP-1 cells and in vivo test methods:

- a) 900  $\mu$ l THP-1 cells ( $0.5 \times 10^6$  cells) together with 100 U  $\gamma$ -interferon/0.9 ml RPMI 1640 medium (containing 2 mM L-glutamine and 5 % heat-inactivated foetal calf serum) are pipetted into 24 well culture plates. 100  $\mu$ l of the compound to be tested are then added. After 3 hours at 37 ° C in 5 % CO<sub>2</sub>/95 % air, 10  $\mu$ l lipopolysaccharide 500  $\mu$ g/ml is added and

the incubation continued for a further 40 hours. Appropriate controls (with and without stimulus, solvent) are also included. The media are then removed and clarified by centrifugation at 1000 g for 10 min. 1.0 ml digitonin 0.01 % is added to the wells to lyse the cells which are loosened by scraping with a rubber policeman and left at 4 ° C for 10 min. Lactate dehydrogenase measurements are then performed immediately and the samples stored at - 20 ° C until the other determinations can be made. The assays are: IL-1 $\beta$  (medium and lysate), IL-6 (medium), TNF- $\alpha$  (medium), PGE<sub>2</sub> (medium and lysate), lactate dehydrogenase (LDH) and DNA (lysates). IL-1 $\beta$ , IL-6 and TNF- $\alpha$  assays are determined using commercially available ELISA kits (Cistron), PGE<sub>2</sub> is measured using a standard RIA and DNA fluorimetrically using DAPI.

In this test, the compounds of the invention selectively inhibit IL-1 $\beta$  release in concentrations from about 0.01 to 100  $\mu$ M. In contrast IL-6, TNF- $\alpha$ , PGE<sub>2</sub> and DNA levels remain substantially unaffected, and the compounds are non-toxic, since LDH release is unchanged. It has for example been determined that compounds of examples 40 and 50 have an IC<sub>50</sub> value (concentration of compound which inhibits to 50% the release of IL-1 $\beta$ ) of 1 and 0.1  $\mu$ M respectively.

b) LPS-Fever

A LPS-suspension (Sigma, No. L-5886; 100 $\mu$ g/5ml glucose solution/kg s.c.) is injected in male Tuttlingen SD rats (150-160g). 2 hours later the body temperature is measured using a thermistor rectal probe connected to an ELLAB telethermometer. After 4 hours the test compound is administered p.o. 2 hours later (6 hrs after LPS administration) the temperature is measured again. The temperature increment shown by the untreated controls is taken as 100% and that in

the treated group is expressed as a percentage of this value. The  $ED_{50}$  is the dose causing a 50% inhibition of the temperature increase determined in the control rats. In this test compounds of the invention inhibit the LPS-induced temperature increase when administered at a dosage in the range of from 0.001 to 0.1 mg/kg p.o. It has for example been determined that compounds of examples 40 and 50 have each an  $ED_{50}$  value of 0.01 mg/kg p.o. and compound of example 51 an  $ED_{50}$  value of 0.05 mg/kg p.o.

c) Carrageenan-Induced Paw Edema in the Rat

50FA male rats, 150-170g body weight, are used for each group. The test compound is administered orally as a suspension in physiological saline/0.5% tragacanth 1 hour prior to the carrageenan injection. Carrageenan (0.1ml of a 1% suspension in physiological saline) is given by subplantar injection into one hind paw. The swelling of the paw is measured by means of an antiphlogometer according to Kemper & Ameln. A control reading is taken immediately after the injection, and the swelling is measured after 3 and 5 hrs. The mean value of the 3- and 5-hour reading is taken after deduction of the control reading, the values obtained from the treated animals are expressed as a percentage of the value obtained from non-treated controls. The  $ED_{50}$  is the dose causing a 50% inhibition of the carrageenan-induced swelling after 3 hrs. In this test method compounds of the invention inhibit significantly the carrageenan-induced swelling when administered p.o. at a dosage in the range of from 0.02 to 5 mg/kg. It has for example been determined that compound of example 40 and 50 have an  $ED_{50}$  value of 0.2 and 1 mg/kg p.o. respectively.

- 32 -

Compounds of the invention are therefore useful for the treatment of disorders with an aetiology associated with or comprising excessive IL-1 $\beta$  release, e.g. in a wide variety of inflammatory states and diseases, for example tissue calcium depletion, degenerative processes in bone and cartilage, e.g. rheumatoid arthritis and osteoarthritis, inflammatory bowel disease, irritable bowel disease, septic shock, psoriasis, asthma, adult respiratory distress syndrome, diabetes type I, osteoporosis of various genesis including e.g. climacteric or post-menopausal osteoporosis as well as osteoporosis consequential to old age, immobilization or trauma, arteriosclerosis and Alzheimer disease.

For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general however, satisfactory results are achieved at daily dosage rates of from about 0.001 to about 100 mg/kg, preferably 0.001 to about 10 mg/kg animal body weight. Suitable daily dosage rates for larger mammals, for example humans, are of the order of from about 0.1 mg to about 1 g/day, conveniently administered once, in divided dosages 2 to 4 x/day, or in sustained release form.

Compounds of Examples 50 and 51 are preferred.

In accordance with the foregoing the present invention also provides:

- a) A method for the treatment of disorders with an aetiology associated with or comprising excessive IL-1 $\beta$  release, e.g. as indicated above in a subject in need thereof, which method comprises administering to said subject an effective amount of a compound of formula I, a physiologically-hydrolysable and -acceptable ester or amide thereof, or a pharmaceutically acceptable salt thereof;

- b) A compound of formula I, a physiologically-hydrolysable and -acceptable ester or amide thereof or a pharmaceutically acceptable salt thereof, for use as a pharmaceutical, for example for use as an agent, e.g. in the method as disclosed above.

The compounds of the invention may be administered by any conventional route, in particular nasally, enterally, preferably orally, e.g. in the form of tablets or capsules, or parenterally e.g. in the form of injectable solutions or suspensions or in a suppository form. Unit dosage forms contain, for example from about 25 µg to 500 mg of a compound of the invention.

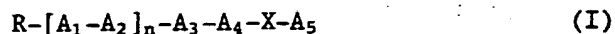
The compounds of the invention may be administered in free form or in pharmaceutically acceptable salt form. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

Furthermore the present invention also provides:

- c) A pharmaceutical composition comprising a compound of formula I, a physiologically-hydrolysable and -acceptable ester or amide thereof or a pharmaceutically acceptable salt thereof, as hereinbefore defined, together with a pharmaceutically acceptable diluent or carrier therefor. Such compositions may be manufactured in conventional manner. They may comprise up to 99.9% by weight of active ingredient.

CLAIM

1. A compound of formula I



wherein

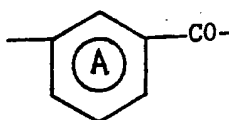
R is hydrogen, an amino protecting group or optionally ring substituted benzyloxy

n is 0 or 1,

A<sub>1</sub> is Val, Leu, Ala, Ile or trimethylsilyl-Ala

A<sub>2</sub> is Phe or Tyr,

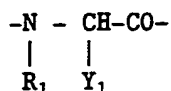
A<sub>3</sub> is a direct bond, Val, Leu, Ala, Ile, trimethylsilyl-Ala or a divalent radical of formula (a)



(a)

wherein ring A is optionally substituted by hydroxy or C<sub>1-4</sub>alkoxy,

A<sub>4</sub> is a direct bond or a divalent radical of formula (b)

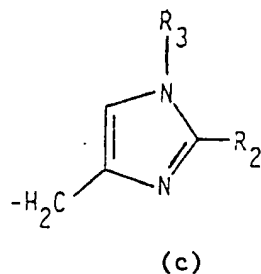


(b)

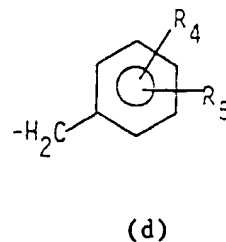
wherein R<sub>1</sub> is hydrogen or C<sub>1-4</sub>alkyl, and

Y<sub>1</sub> is the residue attaching to the α-carbon atom of an α-amino acid and optionally protected, -CH<sub>2</sub>-CH<sub>2</sub>-N(C<sub>1-4</sub>alkyl)<sub>2</sub>, imidazol-2-yl-methyl, benzimidazol-2-yl-methyl, 1H-1,2,4-triazol-3-yl-methyl, pyrazol-3-yl-methyl, indazol-3-yl-methyl or a radical of formula (c) or (d)

- 35 -



or

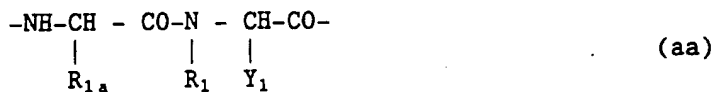


wherein

each of  $R_2$  and  $R_3$ , independently, is hydrogen, halogen,  $C_{1-4}$ alkyl,  $CF_3$  or trityl, at most one of  $R_2$  and  $R_3$  being H, and

each of  $R_4$  and  $R_5$  independently is hydrogen,  $C_{1-4}$ alkyl, hydroxy,  $C_{1-4}$ alkoxy,  $CF_3$ , phenyl or halogen, at most one of  $R_4$  and  $R_5$  being H,

or  $A_3$  and  $A_4$  form together a radical of formula (aa)



wherein  $Y_1$  is as defined above and  $R_1$  and  $R_{1a}$  form together  $-(CH_2)_m-$  wherein  $m$  is 2, 3, 4 or 5, and

i) X is a divalent radical of formula (e<sub>1</sub>)

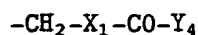


wherein  $R_6$  is H or  $C_{1-4}$ alkyl,

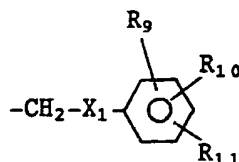
and  $A_5$  is hydrogen;  $CF_3$ ; a radical  $-Z_1-Z_2-Y_2$  wherein each of  $Z_1$  and  $Z_2$  independently is a direct bond or an  $\alpha$ -amino acid residue and  $Y_2$  is  $NH_2$ ,  $C_{1-4}$ alkylamino, di- $(C_{1-4}$ alkyl)amino or a heterocyclic radical attached by a

- 36 -

nitrogen to  $Z_2$ ; a radical  $-\text{CH}_2-\text{X}_1-\text{Y}_3$  wherein  $\text{X}_1$  is O or S and  $\text{Y}_3$  is heteroaryl; a radical  $-\text{CH}_2-\text{Y}_3$ ; or a radical of formulae (k) to (m)

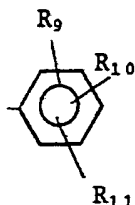


(k)

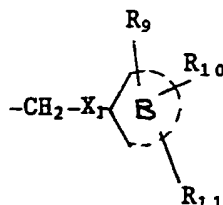


(l)

or



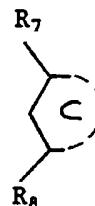
(m)



(o)

wherein

$\text{Y}_4$  is tri-( $\text{C}_{1-4}$ alkyl)methyl or a residue



ring B is pyridyl,

ring C is phenyl or pyridyl,

each of  $\text{R}_7$  and  $\text{R}_8$  independently is  $\text{C}_{1-4}$ alkyl,

$\text{C}_{1-4}$ alkoxy,  $\text{CF}_3$ , halogen, nitro or cyano, and

each of  $\text{R}_9$ ,  $\text{R}_{10}$  and  $\text{R}_{11}$  independently is nitro, cyano,

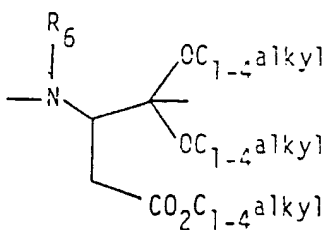
$\text{CF}_3$ , carbamoyl,  $\text{CO}_2\text{R}_{12}$ ,  $-\text{CH}=\text{CH}-\text{CN}$  or  $-\text{CH}=\text{CHCO}_2\text{R}_{12}$

wherein  $\text{R}_{12}$  is  $\text{C}_{1-6}$ alkyl,

X being also a divalent radical of formula ( $\text{e}_2$ )

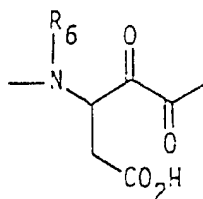


- 37 -

(e<sub>2</sub>)

when A<sub>5</sub> is H, or

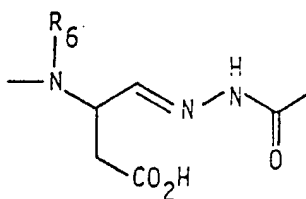
ii) X is a divalent radical of formula (f),



(f)

and A<sub>5</sub> is -Z<sub>1</sub>-Z<sub>2</sub>-Y<sub>2</sub> or a radical of formulae (k) to (o) as defined above, or OR<sub>13</sub> or NR<sub>14</sub>R<sub>15</sub> wherein R<sub>13</sub> is C<sub>1-12</sub>alkyl optionally substituted by OH or interrupted by O and each of R<sub>14</sub> and R<sub>15</sub> is independently hydrogen, C<sub>1-12</sub>alkyl, C<sub>5-7</sub>cycloalkyl or benzyl, or

iii) X is a divalent radical of formula (g)

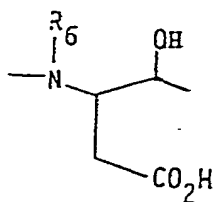


(g)

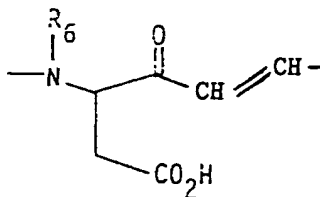
and A<sub>5</sub> is -Z<sub>1</sub>-Z<sub>2</sub>-Y<sub>2</sub> as defined above, or

iv) X is a divalent radical of formula (h) or (j)

- 38 -



(h)



(j)

and  $A_5$  is a radical of formulae (k) to (o),  $-\text{CH}_2-\text{Y}_3$  or  $-\text{CH}_2-\text{X}_1-\text{Y}_3$  as defined above,

with the provisos that

only one of  $A_3$  and  $A_4$  can be a direct bond when  $n$  is 0, and each of  $A_3$  and  $A_4$  is other than a direct bond when  $n$  is 1.

and the physiologically-hydrolysable and -acceptable esters or amides thereof,

in free form, in salt form or in the form of complexes.

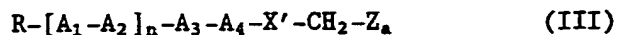
2. A compound of formula I according to claim 1, wherein  $n$  is 0,  $A_3$  is a direct bond, Val, Leu, Ala, Ile or trimethylsilyl-Ala,  $A_4$  is as defined in claim 1, or  $A_3$  and  $A_4$  form together a radical of formula (aa) as defined in claim 1, and
  - i)  $X$  is a radical of formula (e<sub>1</sub>) or (e<sub>2</sub>) as defined in claim 1 and  $A_5$  is H, or
  - ii)  $X$  is a radical of formula (e<sub>1</sub>) or (f) and  $A_5$  is  $-\text{Z}_1-\text{Z}_2-\text{Y}_2$  as defined in claim 1 or a radical of formula (k), (l) or (m) as defined in claim 1 wherein  $X_1$  is 0,
  - iii)  $X$  is a radical of formula (g) and  $A_5$  is  $-\text{Z}_1-\text{Z}_2-\text{Y}_2$  as defined in claim 1, or
  - iv)  $X$  is a radical of formula (j) and  $A_5$  is a radical of formula (k), (l) or (m) as defined in claim 1 wherein  $X_1$  is 0.

and the physiologically-hydrolysable and -acceptable esters or amides thereof, in free form, in salt form or in the form of complexes.

3. A compound of formula I according to claim 1 wherein n is 0 and each of A<sub>3</sub> and A<sub>4</sub> are other than a direct bond, and the physiologically-hydrolysable and -acceptable esters or amides thereof, in free form, in salt form or in the form of complexes.
4. A compound of formula I according to claim 1 or 3 wherein Y<sub>1</sub> is the residue attaching to the  $\alpha$ -carbon atom of an  $\alpha$ -amino acid selected from Ala, Leu, His, Phe, Met, Trp, trimethylsilyl-Ala and optionally side chain protected Arg, Orn and Lys, or a radical of formula (c), and the physiologically-hydrolysable and -acceptable esters or amides thereof, in free form, in salt form or in the form of complexes.
5. A compound of formula I according to claim 1, 3 or 4 wherein X is a radical of formula (e<sub>1</sub>), (h) or (j), A<sub>5</sub> being other than H when X is (e<sub>1</sub>), and the physiologically-hydrolysable and -acceptable esters or amides thereof, in free form, in salt form or in the form of complexes.
6. (Benzyloxycarbonyl-valyl-alanyl)-3R-3-amino-4-oxo-5-(2,6-dichlorobenzoyloxy) pentanoic acid ethyl ester, (benzyloxycarbonyl-valyl-alanyl)-3R,S-3-amino-4-oxo-5-(2,6-dichlorobenzoyloxy) pentanoic acid ethyl ester, (benzyloxycarbonyl-valyl-alanyl)-3R-3-amino-4-oxo-5-(2,6-dichloropyridyl-4-carbonyloxy) pentanoic acid ethyl ester, (benzyloxycarbonyl-valyl-alanyl)-3R,S-3-amino-4-oxo-5-(2,6-dichlorobenzoyloxy) pentanoic acid, (benzyloxycarbonyl-valyl-alanyl)-3R,S-3-amino-4-oxo-5-(2,6-dichlorobenzoyloxy) pentanoic acid isopropyl ester, (benzyloxycarbonyl-valyl-alanyl)-3R,S-3-

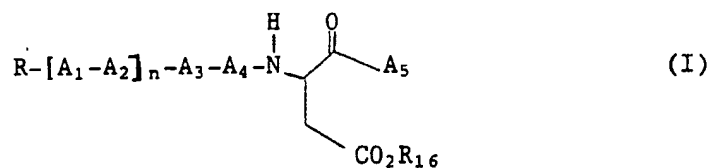
amino-4-oxo-5-(2,6-dichlorobenzoyloxy) pentanoic acid  
t.-butyl ester, and (benzyloxycarbonyl-valyl-alanyl)-3S-  
-3-amino-4-oxo-5-(2,6-dichlorobenzoyloxy) pentanoic acid  
in free form, in salt form or in the form of complexes.

7. A process for producing a compound of formula I and the physiologically-hydrolysable and -acceptable esters and amides thereof, as defined in claim 1, which process comprises
- a) removing at least one protecting group from a compound of formula I in protected form or adding a protecting group R as defined in claim 1 at the N-terminal group of a compound of formula I; or
  - b) converting one compound of formula I into another compound of formula I; or
  - c) coupling together by an amide bond two peptide fragments, each of which contains at least one amino acid in protected or unprotected form and one peptide fragment containing a radical of formula (e<sub>1</sub>) to (j) as defined in claim 1, the peptide fragments being such that a protected or unprotected peptide having the sequence according to formula I above is obtained and, if necessary, removing the protecting group or groups from a compound of formula I in protected form; or
  - d) for the production of a compound of formula I wherein X is a radical of formula (e<sub>1</sub>) or (h) and A<sub>5</sub> is a radical of formula (k), (l) or (o) or -CH<sub>2</sub>-X<sub>1</sub>-Y<sub>3</sub> as defined in claim 1, reacting a compound of formula III

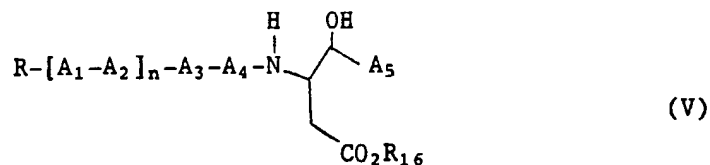


wherein R, A<sub>1</sub> to A<sub>4</sub> and n are as defined in claim 1, X' is a radical of formula (e<sub>1</sub>) or (h) as defined in claim 1, and Z<sub>4</sub> is a leaving group, with a corresponding phenol, thiophenol or HX<sub>1</sub>-pyridine or an acid of formula HX<sub>1</sub>-CO-Y<sub>4</sub> or a functional derivative thereof or HX<sub>1</sub>-Y<sub>3</sub> wherein X<sub>1</sub>, Y<sub>3</sub> and Y<sub>4</sub> are as defined in claim 1; or

e) for the production of a compound of formula I



wherein R, A<sub>1</sub> to A<sub>5</sub> and n are as defined in claim 1 and R<sub>16</sub> is a C<sub>1-12</sub> aliphatic or alicyclic residue, oxidizing a compound of formula V



wherein R, A<sub>1</sub> to A<sub>5</sub>, n and R<sub>16</sub> are as defined above,

and recovering a compound of formula I or a physiologically-hydrolysable and -acceptable ester or amide thereof thus obtained in free or salt form or in the form of a complex.

8. A compound according to any one of claims 1 to 6 for use as a pharmaceutical.
9. A pharmaceutical composition comprising a compound of formula

I as defined in claim 1 or a physiologically-hydrolysable and -acceptable ester or amide thereof, in free form or in physiologically acceptable salt form, together with a pharmaceutically acceptable diluent or carrier therefor.

10. A method for the treatment of disorders with an aetiology associated with or comprising excessive IL-1 $\beta$  release, in a subject in need thereof, which method comprises administering to said subject an effective amount of a compound of formula I as defined in claim 1, a physiologically-hydrolysable and -acceptable ester or amide thereof, or a pharmaceutically acceptable salt thereof.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 92/02472

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C07K5/04; C07K5/06; A61K37/02		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	C07K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	WO,A,9 115 577 (IMMUNEX CORPORATION) 17 October 1991 * See page 5, lines 5-26 * * See page 8, line 6 - page 9, line 37 * ---	1-5,7-10
X	VOELTER ET AL 'Tripeptides and fragments (Volume 3 )' 1983 , GEORG THIEME VERLAG , STUTTGART, GERMANY * See page 180 * ---	1-2,4-5, 7
X	PETTIT ET AL 'Synthetic peptides (Volume 1 )' 1970 , VAN NOSTRAND REINHOOLD COMPANY , NEW YORK, USA * See pages 205 and 211 * --- -/--	1-2,4-5
<p><sup>10</sup> Special categories of cited documents : <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search 11 JANUARY 1993		Date of Mailing of this International Search Report 29. 01. 93
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer KORSNER S.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EDITORS: SMITH ET AL // AUTHORS: WEIDNER ET AL 'Proceedings 12th American Peptide Symposium, June 1991 // Comparison of peptide and protein substrates for interleukin-1beta convertase', ESCOM , LEIDEN, HOLLAND * See pages 891-892 * ---	1-10
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 265, no. 24, August 1990, BALTIMORE, USA pages 14526 - 14528 SLEATH ET AL 'Substrate specificity of the protease that processes human Interleukin-1beta' * See Discussion, pages 14527-8 * -----	1-10



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 92/02472

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 10 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compounds.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO. E**

EP 9202472  
SA 66224

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 11/01/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9115577	17-10-91	AU-A- 7775991	30-10-91